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**WO 01/32030 A1****(54) Title:** FOOD OR VETERINARY COMPOSITION FOR DOMESTIC CARNIVORES CONTAINING FREE TYROSINE**(57) Abstract:** The present invention relates to a food composition for domestic carnivores which makes it possible to prevent or correct pigmentation abnormalities and/or to improve the quality of the fur of the said domestic carnivores, characterized in that it contains a source of free tyrosine representing at least 5 % of the total tyrosine supplied. The invention also relates to a method for preventing or correcting pigmentation abnormalities of the fur of domestic carnivores that uses said food composition.

FOOD OR VETERINARY COMPOSITION FOR DOMESTIC CARNIVORES
CONTAINING FREE TYROSINE

5 The present invention relates to a food composition for domestic carnivores which makes it possible to prevent or correct pigmentation abnormalities and/or to improve the quality of the fur of the said domestic carnivores, characterized in that it contains a source of free tyrosine representing at least 5% of the total tyrosine supplied. The invention also relates to a veterinary composition containing tyrosine,
10 as well as the use of free tyrosine for the preparation of a food and/or veterinary composition intended for preventing pigmentation abnormalities and/or for improving the quality of the fur in a domestic carnivore.

 Despite a rich and nutritionally well-balanced diet, in particular as regards the supply of proteins and amino acids, many breeders or owners of domestic
15 carnivores, particularly dogs and cats, complain of abnormalities in the pigmentation of their animals.

 These "discolorations" cause them substantial financial losses, particularly during competitions, the animals displaying these abnormalities being systematically downgraded or even eliminated by "judges" who know the
20 "standards" of each breed of animal well.

 Domestic carnivores designate dogs and cats, but also other animal species, and particularly animals having a coat, mink, fox, sable and the like.

 These poor pigmentations may occur in the skin, the mucous membranes, the eyes, and more frequently in the hair.

25 The normal colour of these organs is the visual result of the deposits of several sorts of pigments:

- nonmelanic pigments: blood pigments (oxyhaemoglobin, reduced hemoglobin, haemosiderin), carotene and its derivatives;
- melanic pigments or melanins (eumelanins, phaeomelanins, trichochromes) (E. Guaguère et al., Le Point Vétérinaire, 1985, vol. 17,
30 No. 93, 549-557).

 Among these pigments, it is mainly the eumelanins which give the black colour or the dark shade of the pigmentation of the organs.

It is known that the melanins are synthesized by organelles called melanosomes of the specialized cells called melanocytes, from tyrosine, a "semi-essential" aromatic amino acid supplied by the dietary proteins. An amino acid is said to be "essential" when it is necessarily supplied by a source which is external to the animal, because the latter itself does not know how to produce it. Thus, tyrosine is "semi-essential" because the animal can synthesize it from another "essential" aromatic amino acid, phenylalanine. Consequently, for example, the National Research Council (N.R.C.) of the United States of America, which is the world authority on animal nutrition, recommends the following nutritional standards for cats and dogs, based on the dry matter content of the foods, and of which only phenylalanine is considered as being essential (Nutrient Requirements of Cats, 1986; Nutrient Requirements of Dogs, 1985):

	Cats	Dogs
Proteins	240 g/kg (that is 24%)	Not indicated
Phenylalanine + tyrosine	8.5 g/kg (that is 0.85%)	0.72%
Of which phenylalanine	4 g/kg (that is 0.4%)	≥ 50% of Phe + Tyr

These standards have nevertheless currently been widely overtaken by balanced industrial foods which are considerably richer in proteins and in amino acids, and have consequently already a high content of tyrosine.

Yet, in spite of the richness of the current foods supplied by all the major international brands, breeders frequently complain of the pigmentation abnormalities in their animals, and particularly in their fur. Breeders and vets speak in this case of "decolorization", of "discoloration", of "depigmentation", of "dispigmentation", of "red hair", of "yellow hair", of "faded hair", and the like, to designate this syndrome.

The nutritional aetiology of this syndrome is commonly excluded by vets and nutritionists (L. Case et al., Canine and Feline Nutrition, 1995, Mosby Ed., Saint Louis, USA, p 316-319). Particularly, Paragon and Granjean (Rec. Vét. Méd., 1992168(10), 769-77) have excluded a possible deficiency of tyrosine in dogs and cats normally fed in practical conditions.

Only one team in the Ecole Nationale Vétérinaire de Toulouse has described in dogs a "rubra-pilaris syndrome" or red hair disease which can have a nutritional cause (P. Dorchies et al., *Revue de Médecine Vétérinaire*, 1979, 130 (10), 13711382). Indeed, the syndrome is accompanied by an increase in the level of blood and urinary indican. This high presence of indican in the blood and the urine is a sign of a poor digestive use of an essential amino acid, tryptophan, because indican or potassium indoxylsulphate is a metabolite which is synthesized by the bacteria in the large intestine from dietary tryptophan.

The applicant has discovered that this phenomenon of pigmentation abnormality is due to a deficiency in free tyrosine in the diet.

The applicant has indeed discovered that in order to prevent pigmentation abnormalities and/or correct them when they exist in domestic carnivores fed with current balanced foods which have aromatic amino acids, in particular tyrosine, supplied well above the known recommended standards (such as those of N.R.C., for example), it is essential that part of the tyrosine is supplied to the animal in free form and that the tyrosine supply does not exist solely and completely in bound tyrosine form. This discovery is valid regardless of the level of aromatic amino acid contents in the foods, provided that they are equal to or above the recommended nutritional standards.

Conventionally, an amino acid is said to be "bound" when it is embedded in the structure of a protein. To be able to assay a bound amino acid, the protein should be previously and completely hydrolysed, most often with a protease or a strong acid or a strong base. This is the case for the amino acids supplied by the proteins in the dietary raw materials commonly used, whether they are of animal or plant or microbial or fungal origin.

An amino acid is said to be "free" when its assay does not require prior hydrolysis with a protease or a strong acid or a strong base. To assay a free amino acid in a food, it must simply be separated from the structured proteins by precipitating them, with a protein coagulant such as trichloroacetic acid or sulphosalicylic acid for example. After separating the structured proteins, the free amino acid can then be assayed by any known means, such as liquid chromatography for example.

The applicant has discovered, surprisingly, that when at least 5% of the total supplied tyrosine, and still more preferably at least 10%, 15%, 20%, 25% or 30% is supplied to the animal in free form, it is possible to prevent the pigmentation abnormalities in domestic carnivores and/or to treat them when they exist. It is
5 necessary for the free tyrosine to be supplied to the animal, regardless of the quantity of bound tyrosine supplied.

Thus, the invention relates to a domestic carnivore food composition for preventing pigmentation abnormalities and/or improving the quality of the fur, characterized in that it contains:

- 10 - at least one source of proteins of animal or plant origin, and/or
- at least one source of fatty substances of animal or plant origin, and/or
- at least one source of rapid or slow carbohydrates, in particular cereals, and
- 15 - one source of free tyrosine representing at least 5% of the total tyrosine supplied.

In another embodiment of the invention, the free tyrosine level in the food composition will be greater than 10% of the total tyrosine supplied.

In another embodiment, the food composition already contains a level of tyrosine that is well above the nutritional standards as mentioned above.

20 The invention also relates to a domestic carnivore veterinary composition for preventing pigmentation abnormalities and/or improving the quality of the fur, characterized in that it contains a source of free tyrosine representing at least 5%, and preferably at least 10%, 15%, 20%, 25% or 30% of the total tyrosine supplied in the diet.

25 The applicant has therefore developed a method for preventing pigmentation abnormalities and/or improving the quality of the fur in domestic carnivore, characterized in that a quantity of free tyrosine greater than 5%, or preferably 10%, 15%, 20%, 25% or 30% of the quantity of total tyrosine supplied in the diet of the said carnivore is administered to the said carnivore. This process, notably for its
30 esthetic result, is also one of the subjects of the present invention.

The food compositions according to the invention may be dry foods such as biscuits for example, semi-moist foods, for example pates and the like, or moist

foods. These foods also comprise snacks, treats or extruded foods, as well as nutritional supplements.

The veterinary compositions according to the invention include compositions which can be administered by the oral, parenteral or dermal route.

- 5 Preferably, the veterinary composition will be administered by the oral or parenteral route or by the gastrodigestive system. Thus, the veterinary composition according to the invention may be in particular, without this list being limiting, an intragastric, intrainestinal, intramuscular, subcutaneous or intravenous infusion, a hard gelatine capsule, a pill, a soft gelatine capsule, a bolus, a suppository or any other
10 presentation which can be administered by the digestive route.

Conventional excipients may be added to this veterinary composition, such as gelatine, starch, lactose, magnesium stearate, talc, gum arabic and the like.

- The free tyrosine supply may consist of L-tyrosine and/or its salts. Commercially available L-tyrosine may be obtained by extraction after enzymatic
15 or chemical hydrolysis of various animal, plant, microbial or fungal proteins or by any other process known to amino acid producers. It is also possible to use as free tyrosine source a protein which is completely or partially hydrolysed (with a protease, an acid or a base), whether the original protein is of animal, plant, microbial or fungal origin, without the free tyrosine being separated from the rest of
20 the amino acids.

The applicant has also discovered that, particularly in the fur of animals, the supply of free tyrosine improves not only the pigmentation but also the quality of the fur, mainly the density and the length of the hair.

- The examples which follow are intended to illustrate some aspects of the
25 invention without, however, being limiting.

EXAMPLES

Example 1:

- The protein, free tyrosine and bound tyrosine levels of dog and cat foods
30 representative of the well known brands on the world market are assayed. The assays were performed according to the standard methods of the Laréal laboratory, 56250 Saint Nolff, France which is accredited by the French Committee on Accreditation more generally known under the name COFRAC (Accreditation

Number: 1-285). Particularly, the bound tyrosine and the free tyrosine were assayed according to the AFNOR (Association Française de Normalisation) standard method XP V 18-113 (January 1988).

The assays showed that no free tyrosine is present in the commercially available foods. Only bound tyrosine could be assayed.

The results obtained on the crude foods are the following:

Dog foods:

		Proteins (%)	Bound tyrosine (%)
10	Eukanuba Puppy	31.1	1.04
	Eukanuba Junior	26.1	0.85
	Eukanuba Premium	30.3	1.02
	Eukanuba Regular	27.6	0.82
	Hill's Growth	25.6	0.95
15	Hill's Maintenance	22.8	0.87
	Hill's M. Light	16.1	0.57
	Hill's Senior	16.2	0.56
	Proplan Puppy	29.3	1.25
	Proplan Adult	26.5	1.05
20	Proplan Light	26.9	1.02
	Proplan Senior	25.6	0.93
	Royal Canin Mini Junior	32.8	0.92
	Royal Canin Adult 1	27.5	0.83
	Royal Canin Adult 2	27.4	0.80
25	Royal Canin Medium Junior	25.7	0.79
	Royal Canin Medium Adult 1	25.7	0.81
	Royal Canin Medium Adult 2	25.6	0.81
	Royal Canin Maxi Junior	35.7	0.98
	Royal Canin Maxi Adult 1	25.5	0.75
30	Royal Canin Maxi Adult 2	26.2	0.78

Cat foods:

		Proteins (%)	Bound tyrosine (%)
	Iams Chicken	32.6	1.11
	Iams Kitten	34.1	1.06
5	Hill's Growth	34.7	1.29
	Hill's Maintenance	31.2	1.12
	Hill's Senior	29.9	1.23
	Proplan Kitten	33.7	1.23
	Proplan Adult	33.5	1.39
10	Proplan Light	33.9	1.49
	Royal Canin Kitten 34	33.1	1.04
	Royal Canin Sensible 33	33.1	1.07
	Royal Canin Senior 28	28.6	1.11

- 15 These results show that the commercially available dog and cat foods are very rich and widely exceed the standards recommended by N.R.C., mainly for the tyrosine supplies. However, in all these foods, the tyrosine exists completely in the bound form.

20 Example 2:

An experimental complete food for domestic carnivores, presented in the form of extruded foods, and in which the composition of the raw materials is the following (%), was manufactured:

	Animal proteins	31
25	Rice	24
	Maize	5
	Vegetable by-products	13.6
	Fats and oils	16.2
	Flavourings	4
30	Fishmeal	2
	Vitamin-mineral premixes	4
	Commercially available L-tyrosine	0.2
	Total	100

The L-tyrosine provided by the company B.C.F., 56140 Pleucadeuc, France, has a free tyrosine titre of 97%, which means that 0.2% provides 0.19% of free tyrosine in the food.

The analytical characteristics of the food are the following:

5	Proteins	30%
	Fatty substances	22%
	Calcium	0.80%
	Phosphorus	0.75%
	Cellulose	4.50%
10	Metabolizable energy	4340 kcal/kg
	Phenylalanine	1.30%
	Bound tyrosine	1.04%
	Free tyrosine	0.19%
	Total tyrosine (bound + free)	1.23%
15	(Free tyrosine/total tyrosine) × 100 . 15.4%	

This experimental food was distributed to cats on two specialized farms. At the end of the trials which lasted for 6 months on the first farm and 4 months on the second farm, the observations on the fur of the animals, made by specialist breeders and experienced technicians, are the following, with respect to the desired standards for these animals:

Farm	Number of cats	Pigmentation	Qualities of the fur	Length of the hair
M...	5	Very black, without any discoloration	Dense	Long
C...	7	Dark, intense, without any discoloration	Dense, soft to the touch, easy to disentangle	Long

These results show that the experimental food, during many months of trials, prevented any problem of discoloration which is often found on this type of

specialized farms from occurring. In the same manner, the qualities of the fur were judged to have improved compared with cats fed with the previously used foods, which are commercially available foods containing no free tyrosine.

5 Example 3:

A 7-year-old female cat of "Tricolour Persian" breed was fed with commercially available foods containing no free tyrosine. This female cat exhibited abnormalities in its fur compared with the standards for its breed: "faded" black hair and excess of "reddish yellow" hair.

10 The experimental food of Example 2, containing free tyrosine, was given to this female cat. After four and a half months of trial, the "faded hair" become dark and the "reddish yellow" hair defect decreased.

This example shows that a food containing free tyrosine was able to correct the pigmentation abnormalities in a domestic carnivore.

15

Example 4:

Four black-haired cats, were suffering of the "red hair syndrome", while being fed with the commercial diet S9 Felistar® (originating from Royal Canin).

20 Analysis of this food composition shows that it is a balanced food very rich in protein content and that the level of tyrosine is well above the recommended needs for cats:

- Proteins	31 %
- Tyrosine	1.10 %

25 Further analysis of this food shows that the tyrosine is coming exclusively from the structured proteins of the raw materials. The tyrosine present in this food is therefore completely "bound".

After a detailed veterinary examination, these cats received the same food composition, enriched in free tyrosine. The levels of the different forms of tyrosine
30 in this composition are as follow:

- Bound tyrosine	1.10 %
- Free tyrosine	0.43 %
- Total tyrosine	1.53 %

The colors of the cats' coats were as described in the following table:

Cat	Sex	Age (Years)	Color of the coat					
			Day 0	Day 28	Day 59	Day 98	Day 134	Day 212
Charbon	M	9	R	R	R	B	B	B
Jeep	M	6	R	B	B	B	B	B
Iris	F	7	R	B	N.A.	B	B	N.A.
Fida	F	8	R	B	B	B	N.A.	N.A.

M = male, F = female, R = red, B = black, N.A. = non available

5

These results show that addition of free tyrosine in a commercial diet leads to an improvement in the color of the coat of cats suffering from the "red hair syndrome".

CLAIMS

1. Domestic carnivore food composition for preventing pigmentation abnormalities and/or improving the quality of the fur, characterized in that it contains:
 - at least one source of proteins of animal or plant origin, and/or
 - at least one source of fatty substances of animal or plant origin, and/or
 - at least one source of rapid or slow carbohydrates, in particular cereals, and
 - one source of free tyrosine representing at least 5% of the total tyrosine supplied.
2. Food composition according to Claim 1, characterized in that the free tyrosine level is greater than 10% of the total tyrosine supplied.
3. Domestic carnivore veterinary composition for preventing pigmentation abnormalities and/or improving the quality of the fur, characterized in that it contains a source of free tyrosine representing at least 5% of the total tyrosine supplied in the diet.
4. Veterinary composition according to Claim 3, characterized in that the free tyrosine level is greater than 10% of the total tyrosine supplied in the diet.
5. Veterinary composition according to either of Claims 3 and 4, characterized in that it is administered by the oral or parenteral route.
6. Food composition according to either of Claims 1 and 2 or a veterinary composition according to one of Claims 3 to 5, characterized in that the domestic carnivore is chosen from cats, dogs, minks, foxes or sables.
7. Method for preventing pigmentation abnormalities and/or improving the quality of the fur in a domestic carnivore, characterized in that a quantity of free tyrosine greater than 5% of the quantity of total tyrosine supplied in the diet of the said carnivore is administered to the said carnivore.
8. Method according to Claim 7, characterized in that the quantity of free tyrosine is greater than 10% of the quantity of total tyrosine supplied in the diet.
9. Method according to Claim 7 or 8, characterized in that the free tyrosine is administered via a food composition according to one of Claims 1, 2 or 6 and/or a veterinary composition according to one of Claims 3 to 6.

10. Use of free tyrosine for the preparation of a food and/or veterinary composition intended to prevent pigmentation abnormalities and/or to improve the quality of the fur in a domestic carnivore.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23K1/16 A23K1/18 A61K31/195 A61P17/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, BIOSIS, FSTA, CAB Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	FR 2 582 485 A (CORNUAU CLAUDE) 5 December 1986 (1986-12-05) page 1, line 1 - line 13 page 1, line 29 - line 31 page 3, line 28 -page 4, line 6 --- -/--	1,3,5-7, 9,10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 00/01634

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

national Application No

PCT/IB 00/01634

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